High Systolic Blood Pressure Is Associated With Val/Val Genotype in the Catechol-O-Methyltransferase Gene

The Nord-Trøndelag Health Study (HUNT)

Knut Hagen, Elin Pettersen, Lars Jacob Stovner, Frank Skorpen, Jostein Holmen, and John-Anker Zwart

Background: The catechol-O-methyltransferase (COMT) gene contains a functional polymorphism, Val158Met. A few studies on animals have shown a relationship between the COMT gene and BP, but whether this exists in human beings is unclear. The aim of this study was to evaluate the relationship between codon 158 COMT gene polymorphism and BP in a population-based cohort.

Methods: In the 1995–97 Nord-Trøndelag Health Study (HUNT), the association between Val/Met polymorphism at the COMT gene and BP was evaluated in a group of 2966 nondiabetic individuals.

Results: Among the 2591 individuals without current use of antihypertensive drugs, systolic BP >140 mm Hg was more likely among persons with Val/Val genotype compared with the other genotypes (44.8% vs 39.1%, P = .02). In the multivariate analysis the prevalence odds ratio for having the Val/Val genotype was 1.63 (95% CI = 1.18 to 2.24) among individuals with systolic BP ≥160 mm Hg compared with those with systolic BP <140 mm Hg. Val/Val genotype was also more likely (OR = 1.30, 95% CI = 1.04 to 1.63) among individuals with hypertension (as defined by use of antihypertensive medication, systolic BP ≥140 mm Hg, or diastolic BP ≥90 mm Hg) than among those with normal BP.

Conclusions: Based on the study findings, the Val/Val genotype appears to be associated with a higher prevalence of increased systolic BP compared with the Met/Met or Met/Val genotypes at the COMT gene. Am J Hypertens 2007;20:21–26 © 2007 American Journal of Hypertension, Ltd.

Key Words: Blood pressure, polymorphism, Norway, genetics, epidemiology.
an inverse relationship between BP and prevalence of headache\(^\text{10}\) and chronic musculoskeletal complaints,\(^\text{11}\) we asked whether there could be a relationship between BP and the \textit{COMT} gene polymorphism.

In this population-based study performed among unselected adults we evaluated the relationship between codon 158 \textit{COMT} gene polymorphism and BP.

### Material and Methods

#### Study Population

Between August 1995 and June 1997, all inhabitants 20 years or more of age in Nord-Trøndelag county in Norway (\(n = 92,936\)) were invited to participate in the (“Helsuundersøkelsen i Nord-Trøndelag” [HUNT]). In brief, two questionnaires including more than 200 health-related questions were administrated to the participants. The population in Nord-Trøndelag County was ethnically homogeneous (<3% of subjects were of nonwhite ethnicity), making it suitable for epidemiologic genetic research.\(^\text{12}\)

Of the 92,936 individuals invited, 65,291 (70%) answered the first questionnaire and participated in the health examination. Of these, 65,071 answered the question about antihypertensive medication and 64,949 underwent systolic and diastolic BP measurement. Details of the nonparticipants are described elsewhere.\(^\text{12,13}\)

In the HUNT 2 biobank a total of 60,241 DNA samples were available. Genotyping of the \textit{COMT} locus was performed among 3048 individuals. Of these, 69 where excluded because they had self-reported diabetes and 13 because BP data were not available. Among the 2966 individuals who had their BP measured and who did not report diabetes mellitus, approximately 70% were selected completely at random, whereas the remaining 30% had been randomly selected among an older group of individuals who did not have self-reported diabetes mellitus. This latter group was generated in connection with a planned genetic study on diabetes that needed age-matched control subjects for a diabetic population. All age groups were included, but because diabetic patients, as a group, are older than the general population, the total group of 2966 individuals without self-reported diabetes was 3.4 years older (mean 52.5 ± 19.1 years) than the HUNT population as a whole. Of the 2966 individuals, 2960 also answered the questions about vascular diseases, smoking, and physical activity.

#### Measurement of BP

Blood pressure (BP) was measured in the sitting position according to standardized methods described in detail elsewhere.\(^\text{12}\) The BP was measured after a minimum of 2 min rest in the sitting position, and three consecutive standardized BP measurements were recorded at 1-min intervals. In this study the mean of the second and third readings was used.

In the first questionnaire the participants were asked questions about use of antihypertensive medication, smoking status, and level of physical activity. They were also asked to state whether they had histories of diseases or disease conditions such as angina pectoris, myocardial infarction, diabetes mellitus, or stroke.

#### Genotyping of the COMT Locus

Blood sampling was done whenever subjects attended, and details for the procedure and the HUNT 2 biobank are described elsewhere.\(^\text{12}\) The DNA for genotyping was extracted from peripheral blood leukocytes from whole blood or blood clots stored in the HUNT 2 biobank, using the Puregene kit (Gentra Systems Inc., Minneapolis, MN) manually or with an Autopure LS (Gentra Systems Inc.). Laboratory technicians were blinded to the results of the BP measurements. \textit{COMT} genotypes were determined using the LightCycler (Roche Diagnostics Scandinavia AB, Bromma, Sweden) fluorescence resonance energy transfer method.\(^\text{14}\) Polymerase chain reaction (PCR) amplifications were performed in 20-\(\mu\)L reactions on a LightCycler System, using 2 \(\mu\)L genomic DNA and the LightCycler-FastStart DNA Master Hybridization Probes kit (Roche Diagnostics Scandinavia AB, Bromma, Sweden). Table 1 shows PCR primers (Eurogentec, Seraing, Belgium) and fluorescence-labeled probes (PROLIGO, Paris, France). Based on melting curve profiles, participants were classified as having Val/Val, Val/Met, or Met/Met genotypes. Details on PCR and melting curve conditions are available on request.

The study was approved by the Regional Committee for Ethics in Medical Research, and by the Norwegian Data Inspectorate.

#### Statistical Analysis

Differences between continuous variables were tested with analyses of variance (one-way ANOVA) and between dichotomous variables with the \(\chi^2\) test. Analyses used two-tailed estimation of significance, and \(P < .05\) was considered to be statistically significant. The BP values were compared with a nonparametric Kruskal-Wallis test because the BP data were not compatible with a normal distribution evaluated by Shapiro-Wilk \(W\) test.

In the multivariate analyses using multiple logistic regression, we estimated prevalence odds ratios (OR) for the

### Table 1. Primers and hybridization probes used for \textit{COMT} Val158Met genotyping

<table>
<thead>
<tr>
<th>Primers</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probes</td>
<td>5’-ACGCCGTTAGTTCAGGAGCA-3’</td>
<td>5’-GTCTTTTCTCAGCCCGAG-3’</td>
</tr>
<tr>
<td>Sensor</td>
<td>5’-TCAGGCCAGCGAAATCCA-FI-3’</td>
<td>5’ LC Red 640-ATCCGCTGGGTATG GCG-3’</td>
</tr>
</tbody>
</table>

\(\text{Fl} = \text{Fluorescein}; \text{LC Red 640} = \text{Light Cycler Red 640.}\)

Underlined \(C\) indicates polymorphic site.
association between the BP categories and genotype. The precision of the OR was estimated with 95% confidence interval (CI). Systolic BP was divided into three categories: 1) <140 mm Hg; 2) 140 to 159 mm Hg; and 3) ≥160 mm Hg. Diastolic BP was likewise divided into three categories: 1) <90 mm Hg; 2) 90 to 109 mm Hg; and 3) ≥110 mm Hg. To eliminate the influence of antihypertensive medication, individuals who reported current use of such drugs were excluded. We evaluated potential confounding by adjusting for sex, age (5-year categories), years of education (<10, 10 to 12, and >12 years), and body mass index. When appropriate, BP categories were treated as a continuous variable and was incorporated in a two-sided test for trend to evaluate the probability of a linear relation between BP and a specific COMT genotype. We also evaluated the association between the genotypes and hypertension defined as current use of antihypertensive drugs or systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS Inc, Chicago, IL).

## Results

The genotype distribution among the 2966 individuals was in Hardy-Weinberg equilibrium. The demographic data of the different genotype groups are shown in Table 2. The individuals in the three genotype groups did not differ significantly regarding to sex, age, body mass index, cholesterol level, smoking status, level of physical activity, prevalence of stroke, or education level. However, the prevalence of self-reported heart disease (including angina pectoris or myocardial infarction) tended to be lower among individuals with Val/Val genotype than among those with other genotypes (6.4% v 8.7%, P = .08). The individuals with known genotype were significantly older and had, as a consequence, higher median systolic BP and higher mean cholesterol level than those without COMT data available (P < .05) (Table 2).

Although median systolic BP was tended to be highest among those with the Val/Val genotype, neither median systolic or diastolic BP differed significantly across the genotypes. However, the proportion of subjects with systolic BP ≥140 mm Hg tended to be higher among those with Val/Val genotype than among those with other genotypes (49.6% v 45.5%, P = .07), but only evident in the groups of subjects more than 40 years of age (Fig. 1). This difference did not seem to be explained by use of antihypertensive medication, because when we excluded the 375 persons who reported current use of antihypertensive drugs, the proportion with systolic BP ≥140 mm Hg was significantly higher among individuals with Val/Val genotype than among those with other genotypes (44.8% v 39.1%, P = .02).

### Table 2. Clinical characteristics of the subjects according to COMT genotypes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No COMT genotyping (n = 61,983)</th>
<th>Met/Met (n = 946)</th>
<th>Met/Val (n = 1475)</th>
<th>Val/Val (n = 545)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female (%)</td>
<td>53.2 (17.3)*</td>
<td>53.8</td>
<td>55.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>49.1 (17.3)*</td>
<td>52.7 (18.2)</td>
<td>52.5 (18.1)</td>
<td>52.0 (17.9)</td>
</tr>
<tr>
<td>Education &gt; 12 years (%)</td>
<td>19.0</td>
<td>17.4</td>
<td>15.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Median SBP (mm Hg)</td>
<td>134*</td>
<td>137</td>
<td>137</td>
<td>139</td>
</tr>
<tr>
<td>Median DBP (mm Hg)</td>
<td>79</td>
<td>85.5</td>
<td>80</td>
<td>81</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SD)</td>
<td>26.4 (4.1)</td>
<td>26.3 (4.1)</td>
<td>26.4 (4.1)</td>
<td>26.3 (4.2)</td>
</tr>
<tr>
<td>Cholesterol, mmol (SD)</td>
<td>5.89 (1.26)*</td>
<td>6.03 (1.31)</td>
<td>6.03 (1.30)</td>
<td>6.07 (1.37)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>29.0</td>
<td>28.0</td>
<td>29.6</td>
<td>29.0</td>
</tr>
<tr>
<td>High level of physical activity (%)</td>
<td>24.1</td>
<td>20.0</td>
<td>18.8</td>
<td>20.2</td>
</tr>
<tr>
<td>Current use of antihypertensive medication (%)</td>
<td>11.0</td>
<td>12.6</td>
<td>13.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>3.3</td>
<td>3.7</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Angina pectoris (%)</td>
<td>5.0</td>
<td>6.9</td>
<td>7.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>1.9</td>
<td>2.5</td>
<td>1.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>

DBP = diastolic blood pressure; SBP = systolic blood pressure.

* P < .05.
† High level of physical activity was defined as high intensity exercise >1 h per week.

![FIG. 1. Unadjusted prevalence of systolic blood pressure (BP) ≥140 mm Hg by age in individuals with or without the Val/Val genotype (*P = .02).](image-url)
Table 3. Prevalence odds ratio (OR)* for Val/Val genotype related to blood pressure (BP) among 2591 individuals without current use of antihypertensive medication

<table>
<thead>
<tr>
<th>BP (mm Hg)</th>
<th>Total no.</th>
<th>No.</th>
<th>%</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;140</td>
<td>1550</td>
<td>267</td>
<td>17.2</td>
<td>1.00</td>
<td>Ref.</td>
</tr>
<tr>
<td>140–159</td>
<td>625</td>
<td>123</td>
<td>19.7</td>
<td>1.27</td>
<td>(0.98–1.64)</td>
</tr>
<tr>
<td>≥160</td>
<td>416</td>
<td>94</td>
<td>22.6</td>
<td>1.63</td>
<td>(1.18–2.24)</td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;90</td>
<td>2065</td>
<td>376</td>
<td>18.2</td>
<td>1.00</td>
<td>Ref.</td>
</tr>
<tr>
<td>90–109</td>
<td>476</td>
<td>95</td>
<td>20.0</td>
<td>1.14</td>
<td>(0.87–1.48)</td>
</tr>
<tr>
<td>≥110</td>
<td>50</td>
<td>13</td>
<td>26.0</td>
<td>1.68</td>
<td>(0.87–3.26)</td>
</tr>
<tr>
<td>DBP &lt;90 and SBP &lt;140</td>
<td>1492</td>
<td>257</td>
<td>17.2</td>
<td>1.00</td>
<td>Ref.</td>
</tr>
<tr>
<td>DBP ≥90 or SBP ≥140</td>
<td>631</td>
<td>129</td>
<td>20.4</td>
<td>1.31</td>
<td>(1.01–1.69)</td>
</tr>
<tr>
<td>DBP ≥90 and SBP ≥140</td>
<td>468</td>
<td>98</td>
<td>20.9</td>
<td>1.38</td>
<td>(1.03–1.85)</td>
</tr>
</tbody>
</table>

CI = confidence interval; Ref. = reference value; other abbreviations as in Table 2.

* Analyses are adjusted for sex, age, and body mass index.

In the multivariate analysis, excluding those with current use of antihypertensive drugs and adjusting for age and body mass index, there was a trend of higher prevalence of Val/Val genotype with increasing systolic BP that was evident for both sexes (P < .05). Among individuals with systolic BP ≥160 mm Hg, the prevalence OR of Val/Val genotype was 1.63 (95% CI = 1.18 to 2.24) compared with those with systolic BP <140 mm Hg (Table 3). The adjusted OR for Val/Val genotype for women was 1.66 (95% CI = 1.05 to 2.64), and for men 1.60 (95% CI = 1.00 to 2.58).

For diastolic BP there was a nonsignificant trend (P = .12) of higher prevalence of Val/Val genotype with increasing diastolic BP (Table 3). Among individuals with diastolic BP ≥110 mm Hg, the prevalence odds ratio was 1.68 (95% CI = 0.87 to 3.26) as compared with those with diastolic BP <90 mm Hg (Table 3).

Overall, the prevalence OR for Val/Val genotype was 1.38 (95% CI = 1.03 to 1.85) among subjects with systolic BP ≥140 mm Hg and diastolic BP ≥90 mm Hg compared with those with SBP <140 mm Hg and DBP <90 mm Hg (Table 3).

A total of 1474 individuals had as hypertension defined as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg or reported current use of antihypertensive drugs or both. Among these, the prevalence OR of Val/Val genotype was 1.30 (95% CI = 1.04 to 1.63) compared with those with normal BP (systolic BP <140 mm Hg, diastolic BP <90 mm Hg, and without use of antihypertensive treatment).

Discussion

In a population-based group of individuals without self-reported diabetes mellitus and current use of antihypertensive drugs, we evaluated the relationship between BP and Val/Met polymorphism at the COMT gene. The Val/Val genotype was associated with higher prevalence of systolic BP ≥140 mm Hg compared with other genotypes.

This is the first study reporting a statistically significant, although a relatively weak, association between Val/Val genotype and elevated systolic BP in human beings. A potential link between BP and the COMT gene has been previously suggested by studies on COMT gene-disrupted mice and spontaneously hypertensive rats.5–7 In human beings, only one case-control study has focused on the relationship between Val158Met polymorphism and pregnancy-induced hypertension.8 Our interest in the COMT gene was produced mainly because of the previous link to pain perception,9 and the inverse relationship between BP and self-reported pain found in the present population.10,11

The COMT gene is mapped to chromosome 22q11. Chromosome 22q was not identified as a major locus in a recent meta-analysis of genome-wide scans for hypertension and BP in individuals of white ethnicity,15 but one linkage study using more than 400 markers in a total genome scan identified chromosome 22q as one of several chromosomal areas that may be involved in systolic BP regulation.16 Linkage to chromosome 22q was also reported in two other studies evaluating on a genome-wide scale predisposing loci for essential hypertension17 and exercise-induced BP.18

The mechanism behind the association between elevated BP and Val/Val genotype is not clear, and the effect of the COMT enzyme on BP is poorly understood. COMT enzyme inhibitors do not seem to have an effect on BP in control subjects and in Parkinson patients with intact autonomic regulation.19,20 It is possible that the effect on the degradation of catecholamines could be involved since the Val/Val genotype results in a three- to fourfold higher activity of the COMT enzyme. However, at least in the short term, in regard to BP regulation, rapid conversion of...
catecholamines to their metabolites is more likely to decrease than to increase the BP. Thus, other mechanisms are probably involved if modulation of catecholamine degradation is involved in long-term BP regulation.

The Val/Val genotype is also associated with faster catabolism of dopamine. Thus, theoretically the association between Val/Val genotype and elevated systolic BP could also be mediated by dopamine. Dopamine is involved in the BP regulation via several mechanisms, and its role in the elimination of sodium in the kidney is suggested to be important in the long-term control of BP. Therefore, a faster catabolism of dopamine in the kidney could increase the level of sodium and, as a consequence, could increase BP.

Hypertension is a well-known risk factor for vascular diseases, but the clinical relevance of our findings is unclear. However, it is of interest that in a previous study the Met/Met genotype was associated with a decreased risk of myocardial infarction compared with the Val/Val genotype in a hypertensive Swedish population. In apparent contrast, individuals with Val/Val genotype tended to have a lower prevalence of heart diseases than those with other genotypes in the present study (Table 2). One explanation of the different findings could be that the Swedish study was based on clinically confirmed myocardial infarction, whereas the present study used self-reported information about heart diseases. Possibly, individuals with Val/Val genotype in the present study were less likely to self-report myocardial symptoms because of lower levels of pain sensitivity than those with other genotypes. Thus, silent myocardial ischemia could theoretically be more prevalent among individuals with Val/Val genotype than among those with other genotypes.

Three different genetic haplotypes of the COMT gene have been found to be involved in pain perception in a recent case-control study. Thus, whether the other genetic haplotypes of the COMT gene have relevance for blood pressure remains unclear.

The strengths of this work was the fact that the COMT genotyping was performed in a random sample of individuals without self-reported diabetes from an unscreened and genetically homogenous white Norwegian population. A genetically homogenous population reduces the potential for bias in genetic case-control studies involving admixed ethnicities.

In general, confounding by social and behavioral factors is not problematic with regard to polymorphism–disease association studies, but there is relatively high risk that such positive findings may be coincidental. However, in the present study we evaluated the association between BP and a single polymorphism. The risk of a positive finding caused by chance would have been higher if we had evaluated the relationship between BP and multiple polymorphisms throughout the genome. However, the association between Val/Val genotype and elevated systolic BP was not very strong, and because the risk of a positive finding just by chance cannot be ruled out, the evidence from this study must be tested by replication in other populations.

In conclusion, in this population-based study evaluating the relationship between BP and Val/Met polymorphism among 3035 adults, Val/Val genotype was associated with higher prevalence of systolic BP ≥140 mm Hg compared with the Met/Met or Met/Val genotypes.

Acknowledgments
The Nord-Trøndelag Health Study (The HUNT study) is a collaboration between The HUNT Research Centre, Faculty of Medicine, The Norwegian University of Science and Technology (NTNU); Norwegian Institute of Public Health; and the Nord-Trøndelag County Council.

References


